Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific MagJET Genomic DNA Kit

#K2721, #K2722

Read Storage information (p. 4) upon receipt and store kit components appropriately!

www.thermoscientific.com/onebio

#K2721, #K2722
Lot 00000000
Expiry Date 00.0000

CERTIFICATE OF ANALYSIS

Thermo Scientific™ MagJET™ Genomic DNA Kit is qualified by isolating genomic DNA from 5 mg of mouse liver following the protocols outlined in the manual. The quality of purified genomic DNA is evaluated spectrophotometrically and by agarose gel electrophoresis. The purified genomic DNA has an A_{260}/A_{280} ratio of 1.8 ± 0.2. The functional quality of purified DNA is evaluated by digestion with restriction endonucleases.

Quality authorized by: **Jurgita Žilinskienė**

Rev.1 45

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COMPONENTS OF THE KIT

MagJET Genomic DNA Kit	#K2721 96 preps	#K2722 384 preps
Proteinase K	2 × 1.2 mL	8 × 1.2 mL
Digestion Solution for MagJET gDNA Kit	22 mL	90 mL
Lysis Buffer for MagJET gDNA Kit	35 mL	135 mL
MagJET Magnetic Beads	2 × 1.4 mL	10.6 mL
RNase A	2 × 1.2 mL	8 × 1.2 mL
Wash Buffer 1 (conc.) for MagJET gDNA Kit	25 mL	2 × 50 mL
Wash Buffer 2 (conc.) for MagJET gDNA Kit	50 mL	4 × 50 mL
Elution Buffer	30 mL	2 × 30 mL

STORAGE

Proteinase K solution is stable at room temperature as long as the vial remains sealed. After being opened it should be stored at -20°C. When the kit is delivered, remove the RNase A from the package and store at -20°C. MagJET Magnetic Beads should be stored at 4°C. For longer use, it is recommended to aliquot Elution Buffer as 1-2 mL samples and store at -20°C. Other components of the kit should be stored at room temperature (15-25°C).

DESCRIPTION

The MagJET Genomic DNA Kit is designed for fast and efficient purification of genomic DNA from tissue and cell cultures, bacteria and yeast, as well as from human body samples, such as buccal and urogenital swabs, urine, saliva and hair follicles.
The kit utilizes paramagnetic bead technology enabling high yields and robust performance. High binding capacity, uniform particle size, and rapid magnetic response of MagJET magnetic beads makes the technology ideal for high throughput automatic nucleic acid purification, as well as for manual purification for low sample throughput.
The resulting high quality DNA is free of proteins, nucleases and other contaminants or inhibitors, and can be used in a wide range of downstream applications such as PCR, qPCR or other enzymatic reactions. *See* Table 1 for typical genomic DNA yields from various sources.

PRINCIPLE

The MagJET Genomic DNA Kit uses the highly efficient MagJET magnetic particle-based technology for nucleic acid purification. The whole nucleic acid isolation process combines simple steps of sample lysis, DNA binding to the magnetic beads, washing and elution.

Purification protocols optimized for automated KingFisher instruments utilize a high throughput magnetic bead transfer technique where magnetic beads are transferred through different reagent plates containing lysis, binding, washing and elution reagents. This enables high throughput nucleic acid purification and eliminates multiple pipetting steps.

Alternatively, a protocol is available where buffers and other reagents are transferred in each of the protocol steps, while magnetic beads remain captured on the wall of the tube using a magnetic rack. This allows the kit to be used for various throughput applications using a magnetic rack and manual or automated pipetting equipment.

Table 1. Typical genomic DNA yields from various sources.

	Source	Quantity	DNA yield
1	Mouse heart	5 mg	4.5 µg
		20 mg	18-19 µg
2	Mouse tail (homogenized)	5 mg	6 µg
		20 mg	15 µg
3	Mouse tail (cut)	5 mg	1 µg
		20 mg	4-5 µg
4	Mouse liver	5 mg	14-15 µg
		20 mg	50 µg
5	Mouse spleen	5 mg	20 µg
		20 mg	72-75 µg
6	Mouse kidney	5 mg	9-10 µg
		20 mg	35-38 µg
7	Mouse muscle	5 mg	2.5 µg
		20 mg	8-9 µg
8	Mouse brain	5 mg	3 µg
		20 mg	10.5-11 µg
9	Mouse skin	5 mg	5 µg
		20 mg	18-19 µg
10	Mouse lung	5 mg	9-10 µg
		20 mg	36-38 µg
11	Mouse ear	5 mg	9.5 -10 µg
		20 mg	35-37 µg
12	*E. coli* cells	~ 2×10^9 cells	13-17 µg
13	*S. cerevisiae*	~ 10^8 cells	7-11 µg
14	HeLa cells	~ 10^6 cells	7-11 µg
15	Jurkat cells	~ 10^6 cells	6-12 µg
16	COS-7 cells	~ 10^6 cells	3-5 µg
17	Human blood leucocytes	leucocytes collected from 1 mL of blood	13-17 g

IMPORTANT NOTES

- Add the indicated volume of ethanol (96-100%) to **Wash Buffer 1 (conc.) and Wash Buffer 2 (conc.)** prior to first use:

	96 preps		384 preps	
	Wash Buffer 1	**Wash Buffer 2**	**Wash Buffer 1**	**Wash Buffer 2**
Concentrated buffer	25 mL	50 mL	50 mL	50 mL
Ethanol (96-100%)	**75 mL**	**150 mL**	**150 mL**	**150 mL**
Total volume:	100 mL	200 mL	200 mL	200 mL

After preparing each solution, mark the bottle to indicate that this step has been completed.

- Check all solutions in the kit for any salt precipitation before each use. Re-dissolve any precipitates by warming the solution at 37°C, and then equilibrate to room temperature (15-25°C).
- Wear gloves when handling the **Lysis Buffer** and **Wash Buffer 1** as these reagents contain irritants (*see* page 25 for **SAFETY INFORMATION**).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and pipette tips.
- 1.5-2 mL plastic tubes.
- Vortex.
- Microcentrifuge.
- Disposable gloves.
- 96-100% ethanol, molecular biology grade.
- Equipment for sample disruption and homogenization (depending on the method chosen):
  - Mortar and pestle.
  - Homogenizer.
- Automatic magnetic particle processor and consumables.
- Magnetic particle processing rack.

Buffers

- For mammalian cell lysate preparation:
  a) PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4).
  b) 0.15 M NaCl solution.
  c) TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).
- For gram-positive bacteria lysate preparation:
  Gram-positive bacteria lysis buffer (20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100, add lysozyme to 20 mg/mL immediately before use).
- For yeast lysate preparation:
  Yeast lysis buffer (5 mg/mL Zymolyase 20T, 1 M sorbitol, 0.1 M EDTA).
- For red blood cells lysis:
  RBC buffer (10X buffer: 1.68 M NH_4Cl, 2 mM EDTA).

STARTING MATERIAL HANDLING AND STORAGE

- To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples and perform extractions from fresh material, or material that has been immediately frozen and stored at -20°C or -70°C. Appropriate sample storage is essential for reproducibility and high DNA yields. Yields of DNA may vary depending on sample age, type of sample, and storage conditions.
- Yield of DNA purified from different types of tissue depends on efficiency of homogenization when grinding tissues, tails, insects or skin samples in liquid nitrogen using a mortar and pestle. Disruption of tissue or rodent tail samples into small pieces using knives, scissors or homogenizers results in 2-5 times reduced yield of DNA in comparison with grinding in liquid nitrogen. For qualitative and quantitative DNA purification, incubation of tissue lysates with Proteinase K up to 1 or 2 hours is sufficient. Extension of the hydrolysis procedure with Proteinase K up to 6 or 16 hours (overnight) does not increase yield and quality of purified DNA.

PROTOCOL SELECTION GUIDE

The MagJET Genomic DNA Kit provides optimized protocols for genomic DNA purification from different amounts of starting material (up to 10^6 cells and up to 20 mg tissue). The kit is compatible with automated and manual sample processing, allowing low- to high-throughput nucleic acid purification workflow.

The following selection guide summarizes available protocols depending on starting sample quantity, throughput and sample processing type. Automation protocols are optimized for KingFisher Flex and KingFisher Duo instruments.

Transfer the **Tissue_gDNA_Flex** protocol file to the KingFisher Flex or **Tissue_gDNA_Duo** protocol file to the Kingfisher Duo instrument before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.2 User Manual. The protocol files for MagJET Genomic DNA Kit can be found on product web page on www.thermoscientific.com/onebio.

Sample type	Sample quantity	Throughput per run	KingFisher Flex Instrument	KingFisher Duo Instrument	Manual processing	MagJET purification protocol	Page
Mammalian cell culture	up to 10^6 cells	96	●	-	-	**Protocol A**	page 8
		24	-	●	-	**Protocol C**	page 11
		variable	-	-	●	**Protocol F**	page 15
Tissue, rodent tail and insects	up to 20 mg	96	●	-	-	**Protocol B**	page 10
		24	-	●	-	**Protocol D**	page 13
		variable	-	-	●	**Protocol E**	page 14
Bacterial culture	up to 10^9 cells	variable	●	●	●	**Protocol G**	page 15
Yeast culture	up to 10^8 cells	variable	●	●	●	**Protocol H**	page 16
Blood leucocytes	collected from 1 mL of blood	variable	●	●	●	**Protocol I**	page 17
Urine sediments	from 20-25 mL urine	variable	●	●	●	**Protocol J**	page 18
Urogenital swabs	-	variable	●	●	●	**Protocol K**	page 19
Buccal cells	-	variable	●	●	●	**Protocol L**	page 20
Saliva	-	variable	●	●	●	**Protocol M**	page 21
Hair follicles	8-10 follicles	variable	●	●	●	**Protocol N**	page 22
Milk	Up to 0.2 mL	variable	●	●	●	**Protocol O**	Page 23

GENOMIC DNA PURIFICATION PROTOCOLS AND PIPETTING INSRUCTIONS

Protocol A. Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates.

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex as described on page 7.

1. Collect the Cultured Mammalian Cells as follows:
   a) Suspension cells
      Pellet up to 10^6 cells in an appropriate centrifuge tube for 5 min at 300 × g. Discard the supernatant. Rinse the cells once with PBS to remove residual growth medium. Repeat the centrifugation step and discard the supernatant.
   b) Adherent cells
      Remove growth medium from the cells (use up 10^6 cells). Rinse the cells once with PBS to remove residual medium. Remove and discard PBS. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization. Transfer the cells into a microcentrifuge tube (not included) and pellet by centrifugation for 5 min at 300 × g. Discard the supernatant.
2. Resuspend the cells collected in 40 µL of 0.15 M NaCl solution; add 200 µL of **Digestion Solution** and 20 µL of **Proteinase K** Solution. Mix the cell lysate thoroughly by vortexing or pipetting to obtain a uniform suspension. Incubate the sample at 56°C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (20-30 minutes).
3. Obtain four Thermo Scientific Microtiter deep well 96 plates and one Thermo Scientific KingFisher Flex 96 KF plate.
4. Add the following reagents to the plates and leave the plates at room temperature while the **Sample** plate is being prepared.

Plate number	Plate type	Plate name	Content	Volume per well
1	Microtiter deep well 96 plate	Sample	Isopropanol (100%)	400 µL
			Magnetic Beads*	25 µL
2		Wash 1	Wash Buffer 1 (*supplemented with ethanol)*	800 µL
3		Wash 2_1	Wash Buffer 2 (*supplemented with ethanol)*	800 µL
4		Wash 2_2	Wash Buffer 2 (*supplemented with ethanol)*	800 µL
5	KingFisher Flex 96 KF plate	Elution	Elution buffer	150 µL

* Resuspend Magnetic Beads well by vortexing before use.

5. Prepare the sample plate as follows: after cells have been incubated with Proteinase K, add 20 µL of **RNase A** Solution, mix by vortexing then incubate for 10 minutes at room temperature. Add 300 µL of **Lysis Buffer**, vortex 5-10 seconds and transfer lysates to **Sample** plate (prefilled by 400 µL of isopropanol and 25 µL of magnetic beads).
6. Place a Thermo Scientific KingFisher Flex 96 tip comb for deep well magnets on a **Tip Plate** (empty KingFisher Flex 96 KF plate).
7. Start the **Tissue_gDNA_Flex** protocol on the KingFisher Flex 96 and load the plates according to the KingFisher display. After all the plates have been loaded into the instrument, the protocol will begin.
8. When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and turn off the instrument. The purified gDNA is ready for use in downstream applications. Use the purified gDNA immediately or store it at -20°C.

Protocol B. Instructions for genomic DNA purification from up to 20 mg of tissue, rodent tail and insects using KingFisher Flex 96 and Microtiter deep well 96 plates

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex as described on page 7.

1. Grind up to 20 mg of mammalian tissue, 0.5 cm (mouse) tail clip, or up to 20 mg of insect in liquid nitrogen using a mortar and pestle. Alternatively, cut the tissue into small pieces or disrupt it using a homogenizer.
   Note: For spleen and lung tissue use up to 5 mg. High quantities of DNA (2-4 µg/mg tissue) from indicated tissues result in high viscosity of eluates and contamination by magnetic beads. Using 200 µL (instead of 150 µL) Elution Buffer in step 5 is also recommended when isolating DNA from 5 mg spleen or lung tissues.
2. Collect the material into a 2.0 mL microcentrifuge tube (not provided) prefilled by 200 µL of **Digestion Solution** and mix thoroughly by vortexing for 5-10 s. Add 20 µL of **Proteinase K** Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
3. Incubate the sample at 56°C. During incubation vortex the vial occasionally or use a shaking water bath, rocking platform or thermomixer. Suggested optimal incubation times for different types of tissue are provided in the table below.

Type of tissue	Suggested incubation times
Liver	1 hour
Heart	1 hour
Rodent tail	1 hour
Muscle	1 hour
Brain	1 hour
Spleen	2 hours
Lung	2 hours
Kidney	2 hours
Ear	2 hours
Skin	3 hours
Hair follicles	1 hour

4. Proceed to **Step 3** of **Protocol A: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8 for the further purification.

Protocol C. Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Duo protocol file to the KingFisher Duo as described on page 7. Ensure you are using the KingFisher Duo 12-pin magnet head and heating block.

1. Collect the Cultured Mammalian Cells as follows:

   a) Suspension cells
   Pellet up to 10^6 cells in an appropriate centrifuge tube for 5 min at 300 × g. Discard the supernatant. Rinse the cells once with PBS to remove residual growth medium. Repeat the centrifugation step and discard the supernatant.

   b) Adherent cells
   Remove growth medium from the cells (use up 10^6 cells). Rinse the cells once with PBS to remove residual medium. Remove and discard PBS. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization. Transfer the cells into a microcentrifuge tube (not included) and pellet by centrifugation for 5 min at 300 × g. Discard the supernatant.

2. Resuspend the collected cells in 40 µL of 0.15 M NaCl solution; add 200 µL of **Digestion Solution** and 20 µL of **Proteinase K** Solution. Mix the cell lysate thoroughly by vortexing or pipetting to obtain a uniform suspension. Incubate the sample at 56°C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer for 20-30 minutes (until the cells are completely lysed).
3. Obtain one empty Thermo Scientific Microtiter deep well 96 plate and one Thermo Scientific KingFisher Duo elution strip.
4. Add 20 µL of **RNase A** Solution, mix by vortexing then incubate for 10 minutes at room temperature, add 300 µL of **Lysis Buffer**, vortex 5-10 seconds and transfer lysates to **Sample** row (prefilled by 400 µL of isopropanol and 25 µL of magnetic beads).
5. Prepare the **DNA** plate (Microtiter deep well 96 plate) according to the instructions below.

   Add the following reagents to the rows. **Note that row H is reserved for the tip and should be left empty. Note that rows E, F and G are left empty.**

Plate name and type	Row	Row name	Content	Sample/ reagent volume per well
DNA plate Microtiter deep well 96 plate	A	Sample	Lysed sample	580 µL
			Isopropanol (100%)	400 µL
			Magnetic Beads*	25 µL
	B	Wash 1	Wash Buffer 1 (*supplemented with ethanol*)	800 µL
	C	Wash 2_1	Wash Buffer 2 (*supplemented with ethanol*)	800 µL
	D	Wash 2_2	Wash Buffer 2 (*supplemented with ethanol*)	800 µL
	E	Empty	Empty	Empty
	F	Empty	Empty	Empty
	G	Empty	Empty	Empty
	H	Tip comb	12-Tip comb	-

* Resuspend Magnetic Beads well by vortexing before use.

6. Fill the KingFisher Duo elution strip as follows. Make sure that the **Elution Strip** is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and that nuclease free water is pipetted into the correct wells.

Elution** strip	Content	Reagent volume per well
KingFisher Duo elution strip	Elution Buffer	150 µL

7. Place a Thermo Scientific KingFisher Duo 12-tip comb into **row H** on the **DNA** plate.
8. Switch on the KingFisher Duo instrument. Start the **Tissue_DNA_Duo** protocol and load the plate and **Elution Strip** according to the KingFisher display. Ensure that the **Elution Strip** is placed in the correct direction into the elution block and that the perforated end is facing toward the user. The program will start after all plates have been loaded.
9. After the run is completed, remove the plate and and **Elution Strip** according to the instructions on the KingFisher Duo display and turn off the instrument. Transfer the eluate (which contains the purified DNA) to a new, sterile tube and close immediately. Store on ice for immediate use in downstream applications or store at -20°C.

Protocol D. Instructions for genomic DNA purification from up to 20 mg tissue, rodent tail and insects using KingFisher Duo and Microtiter deep well 96 plates

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Duo protocol file to the KingFisher Duo as described on page 7. Ensure you are using the KingFisher Duo 12-pin magnet head and heating block.

1. Grind up to 20 mg of mammalian tissue, 0.5 cm (mouse) tail clip, or up to 20 mg of insect in liquid nitrogen using a mortar and pestle. Alternatively, cut the tissue into small pieces or disrupt it using a homogenizer.

   Note: use up to 5 mg of spleen and lung tissue. High quantities of DNA (2-4 µg/mg tissue) from indicated tissues result in high viscosity of eluates and contamination by magnetic beads. Using 200 µL (instead of 150 µL) Elution Buffer is also recommended in step 5 when isolating DNA from 5 mg spleen or lung tissues

2. Collect the material into a 2.0 mL microcentrifuge tube (not provided) prefilled by 200 µL of **Digestion Solution** and mix thoroughly by vortexing for 5-10 s. Add 20 µL of **Proteinase K** Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

3. Incubate the sample at 56°C. During incubation vortex the vial occasionally or use a shaking water bath, rocking platform or thermomixer. Suggested optimal incubation times for different types of tissue are provided in the table below.

Type of tissue	Suggested incubation times
Liver	1 hour
Heart	1 hour
Rodent tail	1 hour
Muscle	1 hour
Brain	1 hour
Spleen	2 hours
Lung	2 hours
Kidney	2 hours
Ear	2 hours
Skin	3 hours
Hair follicles	1 hour

4. Proceed to **Step 3** of **Protocol C**: **Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 11 for the further purification.

Protocol E. Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and insects.

This protocol is based on transfer of liquids by pipetting through different purification steps rather than magnetic bead transfer as in KingFisher automatic protocols. It allows the kit to be used for various throughput applications using a magnetic rack and manual or automated pipetting equipment. Protocols for different automated pipetting equipment should be optimized for each platform as well as the sample type used. To enable protocol optimization, all buffers are available to purchase separately.

Note: When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.

Step	Procedure
1	Homogenize up to 15 mg of mammalian tissue (use up to 5 mg of spleen and lung tissue), 0.2-0.4 cm rat or mouse tail clip, or up to 20 mg of insect in liquid nitrogen using a mortar and pestle. Alternatively, cut the tissue into small pieces or disrupt it using a homogenizer.
2	Collect the material into a 2.0 mL microcentrifuge tube (not provided) prefilled by 200 µL of **Digestion Solution** and resuspend. Add 20 µL of **Proteinase K** Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
3	Incubate the sample at 56°C until the tissue is completely lysed and no particles remain. During incubation vortex the vial occasionally or use a shaking water bath, rocking platform or thermomixer. Suggested incubation times for different tissue samples are indicated on page 13.
4	Add 20 µL of **RNase A** Solution, mix by vortexing then incubate for 10 min at room temperature. Add 300 µL of **Lysis Buffer**, vortex 5-10 seconds and transfer lysate to a tube prefilled with 400 µL of isopropanol and 25 µL of **magnetic beads** suspension, resuspended well by vortexing. Mix the tube by vortexing.
5	Place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 3 minutes. Remove the supernatant using a pipette.
6	Remove the tube from the magnetic rack and add 800 µL **Wash Buffer 1** (supplemented with ethanol, see p. 5). Resuspend the magnetic beads by vortexing, place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 2-3 minutes. Discard the supernatant by using a pipette.
7	Remove the magnetic rack and add 800 µL **Wash Buffer 2** (supplemented with ethanol, see p. 5). Resuspend the magnetic beads by vortexing, place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 2-3 minutes. Remove the supernatant using a pipette.
8	Repeat step 7 using 800 µL of **Wash Buffer 2.** Make sure that all the supernatant is completely removed in this last washing step. If there are still some droplets visible, spin down the tube, then place it on magnetic rack to collect the beads for 2 minutes and discard remaining supernatant.
9	Remove the magnetic rack and add 150 µL **Elution Buffer**. Resuspend the magnetic beads by vortexing, incubate tubes in thermomixer at 72°C, 600-700 rpm for 5 minutes. Spin down the tube to collect all the drops from the walls of the tube. Place the tube on the magnetic rack and let the magnetic beads collect at the magnet for 2-3 minutes.
10	While on the magnetic rack, transfer the eluate (which contains the purified DNA) to a new clean tube, then close immediately. For long-term storage of DNA, eluting in Elution Buffer and storing at -20°C is recommended.

Protocol F. Instructions for manual genomic DNA purification from up to 10^6 cultured mammalian cells

Note. When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.

Step	Procedure
1	Collection of cultured mammalian cells: a) Suspension cells: collect up to 10^6 cells in a centrifuge tube. Pellet cells by centrifugation for 5 minutes at 250 × g. Discard the supernatant. b) Adherent cells: remove growth medium from a culture plate containing up to 10^6 cells. Detach the cells from the culture plate by trypsinization in an appropriate volume of PBS. Transfer the cells to a microcentrifuge tube and pellet by centrifugation for 5 minutes at 250 × g. Discard the supernatant.
2	Resuspend the cells collected in step 1a or 1b in 40 µL of 0.15 M NaCl solution; add 200 µL of **Digestion Solution** and 20 µL of **Proteinase K** Solution. Mix the cell lysate thoroughly by vortexing or pipetting to obtain a uniform suspension. Incubate the sample at 56°C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer for 20-30 minutes (or until the cells are completely lysed).
3	Add 20 µL of **RNase A** Solution, mix by vortexing then incubate for 10 min at room temperature. Add 300 µL of **Lysis Buffer**, vortex 5-10 seconds and transfer lysate to a tube prefilled with 400 µL of isopropanol and 25 µL of **magnetic beads** suspension, (resuspended well by vortexing). Mix the tube by vortexing, then incubate for 5 min at room temperature.
4	Proceed to **Step 5** of **Protocol E: Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and insects** on page 14.

Protocol G. Instructions for genomic DNA purification from gram-negative bacterial cultures (up to 10^9 cells)

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex or Tissue_gDNA_Duo protocol file to the Kingfisher Duo instrument as described on page 7.

Step	Procedure
1	Harvest up to 10^9 bacterial cells (1 mL of overnight culture) in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 10 min at 5,000 × g. Discard the supernatant.
2	Resuspend the pellet in 40 µL of 0.15 M NaCl solution, add 200 µL of **Digestion Solution** and 20 µL of **Proteinase K** Solution. Mix the cell lysate thoroughly by vortexing or pipetting to obtain a uniform suspension. Incubate the sample at 56°C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (25-30 minutes).
3	For manual gDNA purification, proceed to **Step 4** of **Protocol E: Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and insects** on page 14. For automated purification using KingFisher Flex 96 or KingFisher Duo instruments proceed to **Step 3** of **Protocol A: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8, or **Protocol C: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 11.

Protocol H. Instructions for genomic DNA purification from yeast culture (up to 108 cells)

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex or Tissue_gDNA_Duo protocol file to the Kingfisher Duo instrument as described on page 7.

Before starting:

- Prepare yeast lysis buffer: 1 M sorbitol, 0.1 M EDTA, pH 7.4. Just prior to use add 0.1% β-mercaptoethanol and 5 mg/mL Zymolyase 20T.

Step	Procedure
1	Harvest up to 10^8 yeast cells (about 1 mL of overnight culture) in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 5-10 seconds at maximum speed ≥12,000 × g. Discard the supernatant.
2	Resuspend the pellet in 500 µL of yeast lysis buffer. Incubate for 1 hour at 37°C. Centrifuge cells for 10 minutes at 3,000 × g. Discard the supernatant.
3	Resuspend the pellet in 200 µL of **Digestion Solution**. Add 20 µL of **Proteinase K** Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension. Incubate the sample at 56°C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (45-60 minutes).
4	For manual gDNA purification, proceed to **Step 4** of **Protocol E: Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and insects** on page 14. For automated purification using KingFisher Flex 96 or KingFisher Duo instruments proceed to **Step 3** of **Protocol A: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8, or **Protocol C: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 11.

Protocol I. Instructions for genomic DNA purification from blood leucocytes

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex or Tissue_gDNA_Duo protocol file to the Kingfisher Duo instrument as described on page 7.

Before starting:

- Prepare 1X RBC buffer (10X buffer: 1.68 M NH_4Cl, 2 mM EDTA).

Step	Procedure
1	Add 3 volumes of cold (4°C) prepared 1X RBC buffer to fresh blood (0.5-1 mL) in plastic tube.
2	Mix completely by vortexing and incubate on ice for 4-7 minutes. Mix briefly by vortexing two times during incubation. The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes.
3	Centrifuge at 1,700 × g for 5 minutes at 4°C.
4	Carefully remove and discard the supernatant completely without disturbing the visible white leukocytes pellet.
5	For manual gDNA purification, proceed to **Step 2** of **Protocol E: Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and insects** on page 14. For automated purification using KingFisher Flex 96 or KingFisher Duo instruments proceed to **Step 2** of **Protocol A: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8, or **Protocol C: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 11.

Protocol J. Instructions for genomic DNA purification from urine sediments

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex or Tissue_gDNA_Duo protocol file to the Kingfisher Duo instrument as described on page 7.

Before starting:

- Prepare 1X TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

Step	Procedure
1	Dilute 20-25 mL morning urine by equal volume of **TE buffer** or distilled water to prevent precipitation of salts.
2	Collect cells by centrifugation at 4,000 × g for 30 minutes at room temperature.
3	Discard the supernatant.
4	For manual gDNA purification, proceed to **Step 2** of **Protocol E: Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and isects** on page 14. For automated purification using KingFisher Flex 96 or KingFisher Duo instruments proceed to **Step 2** of **Protocol A: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8, or **Protocol C: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 11.

Protocol K. Instructions for genomic DNA purification from urogenital swabs

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex or Tissue_gDNA_Duo protocol file to the Kingfisher Duo instrument as described on page 7.

Before starting:

- Prepare 1X TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

Step	Procedure
1	A. Swab material collected using a shaft with cotton or brush tips should be placed into a long centrifuge tube (10-12 mL) containing 500 µL TE buffer, slightly vortex for 15-20 minutes and then remove the shafts with tips from tubes. Collect cells by centrifugation at 5,000 × g for 10 minutes at 4°C. B. If urogenital swabs were collected into LBC (liquid based cytology) medium, collect cells for DNA purification from 2 mL of medium by centrifugation at 5,000 × g for 10 minutes at 4°C.
2	Discard the supernatant.
3	For manual gDNA purification, proceed to **Step 2** of **Protocol E: Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and insects** on page 14. For automated purification using KingFisher Flex 96 or KingFisher Duo instruments proceed to **Step 2** of **Protocol A: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8, or **Protocol C: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 11.

Protocol L. Instructions for genomic DNA purification from human buccal cells

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex or Tissue_gDNA_Duo protocol file to the Kingfisher Duo instrument as described on page 7.

Before starting:

- Prepare 1X TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

Step	Procedure
1	There are several buccal cell collection methods: swabs, brushes, mouthwash, and treated cards, such as FTA cards. A. Swab samples obtained using plastic shafts and cotton tips should be immediately placed in a long centrifuge tube (10-12 mL) containing 500 µL TE buffer, thoroughly vortexed for 5 minutes and the swabs removed. Collect cells by centrifugation at 5,000 × g for 10 minutes at 4°C. B. If the swab material will not be used immediately, allow it to dry and then place in a sealed plastic tube. Before DNA purification, place plastic shaft with cotton or brush tips into a long centrifuge tube (10-12 mL) containing 500 µL TE buffer, vortex gently for 10-15 minutes and then remove the shafts with tips from tubes. Collect buccal cells by centrifugation at 5,000 × g for 10 minutes at 4°C. C. Collect mouthwash samples in 50 mL polypropylene conical test tubes and collect cells by centrifugation at 5,000 × g for 10 minutes at 4°C.
2	Discard the supernatant.
3	For manual gDNA purification, proceed to **Step 2** of **Protocol E: Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and insects** on page 14. For automated purification using KingFisher Flex 96 or KingFisher Duo instruments proceed to **Step 2** of **Protocol A: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8, or **Protocol C: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 11.

Protocol M. Instructions for genomic DNA purification from saliva

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described onpage 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex or Tissue_gDNA_Duo protocol file to the Kingfisher Duo instrument as described on page 7.

Before starting:

- Prepare 1X TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

Step	Procedure
1	Collect cells from saliva (diluted by equal volume of TE buffer by centrifugation at 5,000 × g for 10 minutes at room temperature.
2	Discard the supernatant.
3	For manual gDNA purification, proceed to **Step 2** of **Protocol E: Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and insects** on page 14. For automated purification using KingFisher Flex 96 or KingFisher Duo instruments proceed to **Step 2** of **Protocol A: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8, or **Protocol C: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 11.

Protocol N. Instructions for genomic DNA purification from hair follicles

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex or Tissue_gDNA_Duo protocol file to the Kingfisher Duo instrument as described on page 7.

Before starting:

- Prepare 1X TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

Step	Procedure
1	Collect 8-10 hairs with follicles (about 0.5 cm in length) into a 2.0 mL microcentrifuge tube, add 200 µL of Digestion Solution and resuspend.
2	Add 20 µL of Proteinase K Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
3	Incubate the sample at 56°C until the tissue is completely lysed and no particles remain. During incubation vortex the vial occasionally or use a shaking water bath, rocking platform or thermomixer. Suggested incubation times for different tissue samples are indicated on page13.
4	For manual gDNA purification, proceed to **Step 4** of **Protocol E: Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and insects** on page 14 for manual purification. For automated purification using KingFisher Flex 96 or KingFisher Duo instruments proceed to **Step 3** of **Protocol A: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8, or **Protocol C: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 11. **Note.** It is recommended to elute DNA in 50 µL of Elution Buffer.

Protocol O. Instructions for genomic DNA purification from milk

Note:

- When using the MagJET Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the Tissue_gDNA_Flex protocol file to the KingFisher Flex or Tissue_gDNA_Duo protocol file to the Kingfisher Duo instrument as described on page 7.

Step	Procedure
1	Add 300 µL of **Lysis Buffer** to 200 µLof fresh milk sample (without any preservation reagents), vortex 5-10 seconds and add 20 µL of **Proteinase K** solution, mix by vortexing and incubate for 30 min at 56°C.
2	For manual gDNA purification: transfer lysate to a tube prefilled with 400 µL of isopropanol and 25 µL of **magnetic beads** suspension, resuspended well by vortexing and proceed to **Step 5** of **Protocol E: Instructions for manual genomic DNA purification from up to 20 mg tissue, rodent tail and insects** on page 14. For automated purification using KingFisher Flex 96 instruments: transfer lysate to **Sample** plate prefilled with 400 µL of isopropanol and 25 µL of **magnetic beads** suspension and proceed to proceed to **Step 3** of **Protocol A: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 8 (**Note.** Skip treatment with RNase A step described on Step 5). For automated purification using KingFisher Duo instruments: transfer lysate to **Sample** row prefilled with 400 µL of isopropanol and 25 µL of **magnetic beads** suspension according to **Step 5** of **Protocol C: Instructions for genomic DNA purification from up to 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 11.

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified DNA	**Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocols. **Starting material was not completely digested**. Tissue samples should be homogenized very thoroughly in liquid nitrogen. Samples of cells should be suspended very well in small quantity of TE or 0.15 M NaCl solution (30-40 µL) before adding Digestion Solution. **Isopropanol was not added to the lysate.** Make sure that the isopropanol was added to the lysate before transferring the sample to the plate of magnetic particle processor. Magnetic beads have to be resuspended well by vortexing before adding to isopropanol. **Ethanol was not added to Wash Buffer(s).** Make sure that ethanol was added to Wash Buffer 1 and Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on p.5.
Purified DNA is degraded	**Sample was frozen and thawed repeatedly.** Avoid repeated freeze / thaw cycles of the samples. Use a new sample for DNA isolation. Perform extractions from fresh material when possible. **Inappropriate sample storage conditions.** Store mammalian tissues at -70°C and bacteria at -20°C or -70°C until use. For long term storage, clinical samples (urine, saliva) should be stored at -20°C.
RNA contamination	**RNase A treatment was not carried out.** Carry out RNase A treatment step described in the purification procedure.
Low A260:A280 ratio from UV measurement	Some magnetic particles are left in the elution: centrifuge eluates at full speed for 1 minute and transfer supernatant to a new tube.
Inhibition of downstream Enzymatic reactions	**Purified DNA contains residual ethanol.** If residual ethanol is present in eluates of purified DNA, include additional drying step (4-5 minutes at room temperature) before the elution step into DNA purification program of magnetic particle processors. The instructions can be found in the BindIt Software for KingFisher Instruments version 3.2 User Manual. **Purified DNA contains residual salt.** Use the correct order of the Wash Buffers. Always wash the magnetic beads with Wash Buffer 1 first and then proceed with Wash Buffer 2.
Carryover of the magnetic beads (MB) in the elution	Carryover of MB in the eluted DNA will not affect downstream applications. To remove the carryover MB from eluted DNA, simply magnetize the MB and carefully transfer to a new tube or plate.

SAFETY INFORMATION

Lysis Buffer for MagJET gDNA Kit

Xn Harmful
Hazard-determining components of labelling: guanidinium chloride
Risk phrases
22 Harmful if swallowed.
36/38 Irritating to eyes and skin.
Safety phrases
23 Do not breathe gas/fumes/vapour/spray.
26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
36/37 Wear suitable protective clothing and gloves.
60 This material and its container must be disposed of as hazardous waste.

Wash Buffer 1 (conc.) for MagJET gDNA Kit

Xn Harmful
Hazard-determining components of labelling: guanidinium chloride
Risk phrases
22 Harmful if swallowed.
36/38 Irritating to eyes and skin.
Safety phrases
3 Keep in a cool place.
23 Do not breathe gas/fumes/vapour/spray.
26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
36/37 Wear suitable protective clothing and gloves.
60 This material and its container must be disposed of as hazardous waste.

Proteinase K

Xn Harmful
Hazard-determining components of labelling: Proteinase, Tritirachium album serine
Risk phrases
R42 May cause sensitisation by inhalation.
Safety phrases
23 Do not breathe gas/fumes/vapour/spray.
36 Wear suitable protective clothing.
45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
60 This material and its container must be disposed of as hazardous waste.

PRODUCT USE LIMITATION
This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.